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Hyperinsulinemia Accompanying Hyperglycemia in Chinese Patients With Aplastic Anemia

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Serum insulin, and plasma glucagon and glucose levels were measured in 56 Chinese patients with aplastic anemia (AA) and 40 normal controls. Serum insulin and plasma glucose levels in 18 newly diagnosed cases and 11 previously treated cases with prednisone were significantly higher than those in the controls. Serum insulin and plasma glucose levels in 27 cases previously treated with stanozolol were significantly higher than those in the newly diagnosed cases and the previously treated cases with prednisone. There was no significant difference in plasma glucagon levels between the patients and the controls. Serum insulin and plasma glucose levels were significantly correlated with the amount of blood transfusions and serum ferritin and cortisone concentrations in the AA patients. Our findings suggest that AA patients may have hyperinsulinemia accompanying hyperglycemia, which can be further aggravated by stanozolol and prednisone therapy and iron overload. Am. J. Hematol. 56:151–154, 1997. © 1997 Wiley-Liss, Inc.

Key words: aplastic anemia; hyperinsulinemia; hyperglycemia

INTRODUCTION

Previous studies have shown that androgen therapy is strongly associated with abnormal glucose metabolism in patients with aplastic anemia (AA) [1]. Multiple blood transfusions leading to transfusional hemochromatosis are important factors related to diabetes mellitus [2,3]. Glucocorticoid administration may also be an important factor of developing abnormal glucose tolerance [4–6]. However, there have been reports that AA and diabetes mellitus may coexist in the absence of treatments [7], which suggests that abnormal glucose tolerance is very likely to be related to AA itself. In order to evaluate the glucose metabolism in AA patients, serum insulin, and plasma glucagon and glucose levels were determined in 56 Chinese patients with AA and 40 nondiabetic controls.

MATERIALS AND METHODS

Subjects

Fifty-six patients (36 males and 20 females) were studied. Their ages ranged from 13 to 66 years. The diagnosis and the type of AA were based on the criteria formulated by the Fourth Chinese Aplastic Anemia Symposium held in 1987 in Baoji, China [8]. All the patients selected to participate in the study belonged in chronic type. They were divided into three groups. Group 1 consisted of 18 newly diagnosed cases. Group 2 was 11 cases who had received prednisone treatment before the study at a dose of 15 to 40 mg/m^2 daily for 1 to 2 months. Group 3 included 27 cases previously treated with stanozolol at a dose of 3 to 6 mg/m^2 daily for 3 to 12 months. No patients in the study had clinical evidence of pancreatic diseases. None had fever or complications of infectious diseases during the study. Body mass index (BMI) was calculated at time of study. The newly diagnosed AA patients had no the transfusion history. The AA patients with the family history of diabetes mellitus were excluded from the study. Blood cell counts, bone marrow aspirates, and bone marrow biopsies were examined in all the patients. We also examined serum albumin (Alb), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), creatinine (Cr), and C-reactive protein

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(CRP). The normal control group was composed of 40 healthy volunteers (25 males and 15 females). Their ages ranged from 16 to 61 years. There were not any hematological diseases, diabetes mellitus, or pancreatic diseases in the controls whose liver and kidney functions were normal.

Determination of Serum Insulin and Plasma Glucagon and Glucose Levels

None of patients had been treated with insulin or any other antidiabetic drugs before the test. In all the subjects venous blood was collected in the morning after an overnight fast (12 h). Serum or plasma was separated from venous blood or anticoagulant venous blood by centrifugation. Plasma glucose concentrations were determined by glucose oxidase method, and serum insulin and plasma glucagon measured by radioimmunoassay. (The assay kits were provided by the Institute of Biologicals of Shanghai.) Serum cortisone and ferritin levels were also measured by radioimmunoassay. (The assay kits were provided by Institute of Atomic Energy of Bejing.)

At the same time, an oral glucose tolerance test (OGTT) was conducted in 18 newly diagnosed cases and 40 normal controls. After a 75-g oral glucose load was given, venous blood was collected at 0, 30, 60, 90, and 120 min. Serum insulin and plasma glucose were determined at each time point.

STATISTICAL ANALYSES

All data are expressed as means ± SD. Comparisons of two groups were made with two-tailed Student's unpaired *t*-tests and comparisons among three or more groups with analysis of variance. Correlations between variables were examined by linear correlation and regression analysis.

RESULTS

Fifty-six patients with AA were similar in age and sex to 40 normal controls. Mean BMI didn't differ significantly ($P > 0.05$) between the AA patients and the controls. Clinical data for three groups of AA patients and control data are summarized in Table I. There was no statistical difference in age or BMI among the three groups of AA patients. The three groups of patients didn't differ significantly ($P > 0.05$) in CRP, blood cell counts, and bone marrow findings, but the disease course of the previously treated cases was longer than that of the newly diagnosed cases.

Serum insulin and plasma glucagon and glucose levels in patients with AA and normal controls are presented in Table II. Serum insulin and plasma glucose levels in all three groups of the AA patients were significantly higher than those in the controls. Of the patients with AA, serum insulin and plasma glucose levels in 27 cases previously treated with stanozolol (Group 3) were significantly ($P < 0.01$) higher than those in 18 newly diagnosed cases (Group 1) and 11 cases previously treated with prednisone (Group 2). There was no significant difference in serum insulin or plasma glucose concentrations between Group 1 and Group 2 ($P > 0.05$). Plasma glucagon concentrations of patients were in the normal range (they were slightly increased in Group 2 and Group 3, but there was no significant difference statistically as compared with the controls).

In Group 3, 7 cases were blood-transfusion-dependent (the accumulated amount of blood transfusions was above 6 l) and 5 cases had hepatic dysfunctions (serum GPT levels were above 100 Karmen U). There was no significant difference ($P > 0.05$) in serum insulin levels between the blood-transfusion-dependency cases (19.47 ± 2.64 mU/l) and 9 cases without transfusion (18.55 ± 2.77 mU/l), but plasma glucose levels (5.89 ± 0.31 mmol/l) in the blood-transfusion-dependency cases were significantly ($P < 0.05$) higher than those (5.34 ± 0.38 mmol/l) in the cases without transfusion. Serum insulin and plasma glucose concentrations in 5 cases with liver dysfunctions were close to those in the cases without liver dysfunctions ($P > 0.05$).

After a 75-g oral glucose load, serum insulin and plasma glucose levels at all time points in 18 newly diagnosed patients and 40 normal controls are depicted in Figures 1 and 2.

Correlations of serum insulin and plasma glucagon and glucose levels with serum ferritin and cortisone concentrations are presented in Table III.

DISCUSSION

Our results showed that serum insulin and plasma glucose levels in 18 newly diagnosed patients with AA were significantly higher than those in the controls, and after a 75-g oral glucose load serum insulin and plasma glucose levels of the newly diagnosed cases at 30, 60, 90, and 120 min were also markedly higher. The newly diagnosed AA patients had no family history of diabetes mellitus, did not take any medications known to interfere with glucose tolerance at the time of study, had no history of pancreatic diseases, did not have any complications of infectious diseases during the study, but had normal CRP levels and normal liver and renal functions. In addition, none of the 18 patients were obese individuals and mean BMI did not differ between the patients and the controls. Therefore, changes of serum insulin and plasma glucose may be associated with AA itself.

It is not clear why serum insulin and plasma glucose levels were markedly increased in AA patients. Olefsky suggested that the presence of hyperinsulinemia accompanying hyperglycemia was related to some degree of

TABLE I. Clinical Data for Three Groups of Patients With AA and Control Data*

	Group 1 (n = 18)	Group 2 (n = 11)	Group 3 (n = 27)	Controls (n = 40)
Sex (male/female)	12/6	7/4	17/10	25/15
Age (year)	29.81 ± 6.24	33.40 ± 7.85	34.03 ± 9.21	33.27 ± 8.18
BMI (kg/m^2)	22.70 ± 2.82	22.46 ± 2.08	23.00 ± 3.02	22.63 ± 2.36
Course of disease (months)	2.43 ± 1.56	29.30 ± 8.74	34.45 ± 10.96	—
CRP (mg/l)	9.39 ± 5.21	8.83 ± 4.86	11.97 ± 5.05	—
Splenomegaly	0/18	0/11	0/27	0/40
GPT (>25 U)	0/18	0/11	5/27	0/40
Alb (<35 g/1)	0/18	0/11	0/27	0/40
Positive HBsAg	1/18	1/11	4/27	0/40
Positive HBeAg	0/18	0/11	0/27	0/40
Positive anti-HBe antibody	0/18	0/11	1/27	0/40
BUN (>7 mmol/1)	0/18	0/11	0/27	0/40
Cr (>100 μmol/1)	0/18	0/11	0/27	0/40
Blood				
Hb (g/l)	52.74 ± 15.53	58.60 ± 16.71	61.08 ± 16.39	122.45 ± 23.17
WBC (×10^9/l)	2.30 ± 1.14	3.11 ± 0.95	2.75 ± 1.27	5.84 ± 1.70
Platelets (×10^9/l)	40.36 ± 8.72	38.27 ± 12.94	42.92 ± 11.58	169.52 ± 37.91
Reticulocytes (%)[a]	0.34 ± 0.12	0.33 ± 0.09	0.40 ± 0.13	1.20 ± 0.36
MCV (μm^3)				
<82	2/18	2/11	1/27	4/40
83–94	15/18	9/11	21/27	33/40
>95	1/18	0/11	5/27	3/40
Positive Ham's test	0/18	0/11	0/27	0/22
Positive Coombs' test	0/18	0/11	0/27	0/17
High NAP score	15/18	8/11	23/27	—
BM aspirate				
RBC series (%)	6.72 ± 2.96	9.28 ± 5.12	8.54 ± 4.42	22.74 ± 7.05[b]
WBC series (%)	12.63 ± 5.73	14.90 ± 6.29	15.35 ± 5.85	53.30 ± 12.81[b]
Nonmyeloid BM cells (%)	80.22 ± 16.09	75.74 ± 13.08	76.03 ± 13.71	23.76 ± 8.59[b]
BM biopsy				
Hypocellularity	17/18	11/11	22/27	—
Increase in reticulin	0/18	0/11	0/27	—

*MCV, mean corpuscular volume; NAP, neutrophil alkaline phosphatase; BM, bone marrow.
[a]Corrected reticulocytes.
[b]n = 8.

TABLE II. Serum Insulin and Plasma Glucagon and Glucose Levels in AA Patients and Controls (Means ± SD)

Group	No. of cases	Serum insulin (mU/l)	Plasma glucagon (ng/l)	Plasma glucose (mmol/l)
1	18	15.24 ± 3.06**	90.73 ± 24.36	5.01 ± 0.37*
2	11	15.53 ± 2.71**	102.34 ± 28.58	5.16 ± 0.40**
3	27	18.67 ± 3.10***	97.29 ± 31.18	5.64 ± 0.43***
Controls	40	12.80 ± 2.22	84.96 ± 26.87	4.71 ± 0.40

*P < 0.05, **P < 0.01, ***P < 0.001, compared with controls.

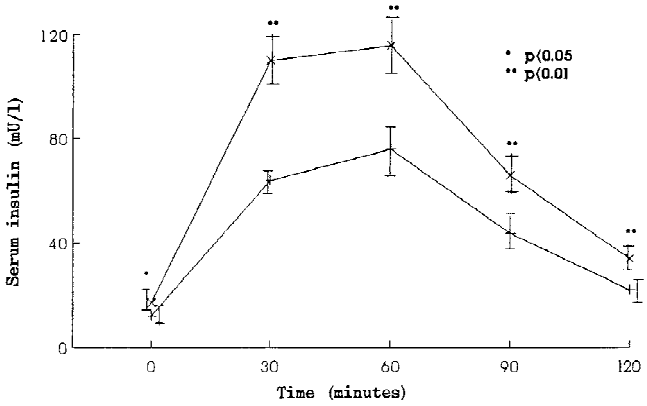

Fig. 1. After a 75-g oral glucose load, serum insulin levels at all time points. × and + indicate 18 newly diagnosed AA patients and 40 normal controls, respectively.

insulin resistance [9]. In addition, our study demonstrated that the highest values of serum insulin and plasma glucose after a 75-g oral glucose load in AA patients were significantly higher than those in normal controls. The increases in ''the highest values'' also indicated the presence of peripheral insulin resistance [10]. We postulated that insulin receptor and postreceptor abnormalities might be present in AA patients, which can lead to insulin resistance.

Androgen is strongly associated with peripheral glucose metabolism [11] or diabetes mellitus [12]. In our study, serum insulin and plasma glucose levels in the AA

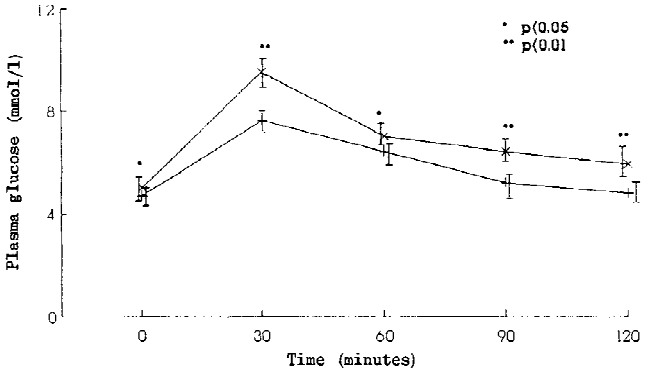

Fig. 2. After a 75-g oral glucose load, plasma glucose levels at all time points. × and + indicate 18 newly diagnosed AA patients and 40 normal controls, respectively.

TABLE III. Correlations of Glucose Metabolism Parameters With Ferritin and Cortisone in 56 AA Patients*

	Serum insulin	Plasma glucagon	Plasma glucose
Serum ferritin	r 0.587	r 0.013	r 0.385
	P <0.001	P >0.05	P <0.01
Serum cortisone	r 0.272	r −0.119	r 0.403
	P <0.05	P >0.05	P <0.01

*r, correlation coefficient; P, probability; $P < 0.05$, there is a significant correlation between two variables. $P > 0.05$, there is no significant correlation between two variables.

patients treated with stanozolol were markedly higher than those in the newly diagnosed AA patients, which was in correspondence with the previous report [1]. A lot of blood transfusions may develop iron overload resulting in secondary diabetes mellitus [13]. We found that plasma glucose levels in transfusion-dependency cases were markedly increased, and serum insulin and plasma glucose levels were positively correlated with serum ferritin concentrations, which indicated that iron overload was also a factor influencing serum insulin and plasma glucose levels in AA. It has been shown that hyperinsulinemia and abnormal glucose tolerance may be present in patients with liver dysfunctions [14,15], but as stated above, serum insulin and plasma glucose levels in 5 cases with liver dysfunctions were not significantly different from those in the cases with normal liver functions. Maybe liver dysfunctions in the 5 AA patients were not very serious. Although we did not find significant differences in serum insulin and plasma glucose concentrations between the cases treated with prednisone and the newly diagnosed cases, serum insulin and plasma glucose levels were positively correlated with serum cortisone concentrations. Therefore, prednisone therapy may further influence glucose metabolism in AA.

In conclusion, our findings suggest that AA patients may have hyperinsulinemia accompanying hyperglycemia, which can be aggravated by stanozolol and prednisone therapy and multiple blood transfusions. The mechanism of developing hyperinsulinemia and hyperglycemia may be peripheral insulin resistance resulting from chronic aplastic anemia.

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